was cyclosporine with four doses of methotrexate in 97 patients and other treatments in 5. Overall survival at 5 years was 61% and relapse-free survival was 56%. The incidence of acute GvHD grades II-IV was 62% in patients with an LC30 of $<0.2 \times 10^9$/L, 33% if the LC30 was 0.2–1.0 $\times 10^9$/L and 25% in patients with an LC30 $>1.0 \times 10^9$/L ($p = 0.008$). Transplant related mortality (TRM) was 34% in patients with an LC30 $<0.2 \times 10^9$ versus 19% (LC30 of 0.2–1.0 $\times 10^9$) and 0% (LC30 $>1.0 \times 10^9$) ($p<0.001$). Survival was significantly higher in 17 patients with an LC30 $>1.0 \times 10^9$/L, compared to 67 patients with an LC30 0.2–1.0 $\times 10^9$/L, and 18 patients with $<0.2 \times 10^9$/L (91% vs. 60%, vs. 36% $p = 0.02$ and 0.001 respectively). When analyzed as a continuous variable in multivariate analysis, a higher LC30 was associated with a lower incidence of acute GvHD grades II-IV, improved survival, less relapse and higher relapse-free survival. Plasma levels of cytokines were measured in 15 subjects between day 12–32 post-transplant (total 21 samples). Six patients had a low ($<0.2 \times 10^9$/L) and 9 patients a high ($>1.0 \times 10^9$/L) LC30. Plasma IL-15 was lower in patients with high LC30 (median 32 pg/ml vs. 56.5 pg/ml, $p <0.05$ log rank sum). These results indicate that the LC30 is a robust prognostic factor for transplant outcome in matched unrelated as well as matched related SCT for myeloid malignancies receiving either BM or PBSC with or without irradiation conditioning. Further research to identify the transplant conditions leading to prompt lymphocyte recovery might lead to global improvements in SCT outcome in unrelated SCT.

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ADOPTIVE TRANSFER OF NKT CELLS REDUCES GVHD SEVERITY VIA AN IFN-Γ AND IL-4 DEPENDENT MECHANISM

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NKT cells, which are CD1d reactive, are thought to play an immunoregulatory role in suppressing dysfunctional immune reactions, including graft-versus-host disease (GVHD). However, we do not know if non-manipulated donor-type NKT can suppress GVHD or how NKT proliferate and migrate following hematopoetic cell transplantation (HCT). We transferred 5.5×10^5 highly purified (>95%) NKT (DX5+TCR+CD4+) from luciferase positive (*luc*+) C57BL/6 (H-2^b) mice into lethally irradiated Balb/c (H-2^d) recipients with 5×10^6 T-cell depleted bone marrow (TCD-BM) from wild-type (WT) C57BL/6 mice, and monitored them by bioluminescence imaging (BLI). By day 4 after transfer, a signal was observed in spleen and lymph node (LN) sites, and between days 7 and 10, NKT had also migrated to skin. Total photons emitted peaked around day 25 after transplantation, followed by a steady decline. To assess the impact of donor-type NKT on GVHD induction by Tcon, we co-transferred various doses of highly pure WT NKT at day 0 with 5×10^6 TCD-BM, followed by 5×10^5 *luc*+Tcon at day 2. We have found that adoptive transfer of as few as 2.5×10^4 NKT can significantly improve survival of mice receiving 5×10^5 Tcon. Survival with Tcon only was 20% and for Tcon with NKT was 74%; $p = 0.0023$. To determine how NKT reduce GVHD, we examined intracellular levels of various cytokines in Tcon with or without 2.5×10^4 NKT, following HCT. At 8 days after HCT, mice receiving NKT had fewer TNFα-positive cells from LNs (CD4: 45% to 27%; CD8 36% to 24%); by day 11, however, TNFα levels between groups were equivalent. IFN-γ levels, which were high in both NKT treated and untreated groups at day 8 (85%-95%), decreased significantly in NKT treated mice by day 11 (CD4: 40%; CD8: 43%), but were abundant in Tcon only mice (CD4: 78%; CD8: 80%) ($p = .0001$). NKT from both IL-4 -/- and IFN-γ -/- mice were less effective at suppressing GVHD than WT NKT, implicating these cytokines in the suppressive mechanism. Finally, we found that NKT do not have a major impact on the graft-versus-tumor effect of Tcon against a *luc*+BCL-1 tumor. These studies indicate that NKT persist *in vivo* upon adoptive transfer and suppress GVHD, even at extremely low cell numbers, which is important given the relative paucity of this cell population. The mechanisms of GVHD suppression appear to be distinct to those of CD4+CD25+FoxP3+ Treg and involve the production of IL-4 and IFN-γ by NKT and a decrease in Tcon which produce pro-inflamatory cytokines.

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CEACAM1 REGULATES EXPERIMENTAL GRAFT-VERSUS-HOST-DISEASE

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Carcinoembryonic antigen associated cell adhesion molecule 1 (Ceacam1) is a type I transmembrane glycoprotein that regulates numerous processes including bacterial colonization of the gastrointestinal lumen and leukocyte function. Ceacam1 is expressed on gut epithelium and activated T cells, particularly in the intestines. We found that T cells from Ceacam1-/- mice had elevated phosphorylation of STAT3. Upon stimulation with anti-CD3+CD28, IL-2, IL-4, IL-6, or IL-12 in vitro, Ceacam1-/- T cells showed hyperphosphorylation of corresponding canonical STAT proteins, indicating that Ceacam1 can regulate the sensitivity of T cells to cytokines and TCR stimulation. Ceacam1-/- T cells also had defective anergy induction as measured by IL-2 secretion. This was associated with decreased cleaved caspase 3 (but not decreased apoptosis) and hypophosphorylation of STAT1 upon IFNγ treatment. We assessed Ceacam1 regulation of T cells in vivo during GVHD. Ceacam1-/- T cells caused increased mortality and large intestinal GVHD, had more profound activation (CD25, CD62L) and expression of integrin α4β7 and demonstrated selective infiltration of the intestines. By contrast, Ceacam1-overexpressing T cells caused significantly less GVHD mortality and damage to all target organs, which correlated with decreased proliferation and organ infiltration. We also studied Ceacam1 in recipients of allogeneic bone marrow transplantation (allo-BMT), and found that compared to wildtype (WT) recipients, Ceacam1-/- recipients with GVHD showed accelerated mortality and increased damage to the large intestines and thymus. Donor alloactivated CD4 T cells in Ceacam1-/- allo-BMT recipients had increased activation (CD25), and trafficked preferentially to the mesenteric lymph nodes, small and large bowel, but had decreased accumulation in the liver and peripheral lymph nodes (PLN). Finally, Ceacam1-/- mice were more sensitive to radiation injury as demonstrated by accelerated kinetics of mortality and decreased numbers of surviving or regenerating small intestinal crypts. We conclude that Ceacam1 is an important regulator of alloreactivity during GVHD through its effects on T cell (allo)activation, proliferation, trafficking, cytokine sensitivity, and anergy. Moreover, Ceacam1 expression on gut epithelium regulates sensitivity to radiation injury, T cell alloreactivity, and intestinal GVHD.

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ELAFIN IS A BIOMARKER OF GRAFT VERSUS HOST DISEASE OF THE SKIN

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There are no plasma biomarkers specific to any of the three target organs of acute graft versus host disease (GVHD): skin, GI tract and liver. We sought to identify a biomarker for skin GVHD in an initial discovery step using an intact proteomic analysis system. We compared plasma pooled from ten patients with skin GVHD only (sGVHD) to ten patients without GVHD (–GVHD) to ten patients with GI tract GVHD only (giGVHD). Of four candidate proteins that were both significantly elevated only in the plasma of sGVHD and that could be measured by ELISA, we selected elafin, an epidermal proteinase inhibitor that is induced by TNF-α and found in inflamed epidermis. We measured levels of elafin